Observations on the Fate of Experimentally Induced Wounds on Adult Chinook Salmon in Sea Water

G. R. Bell and J. W. Bagshaw

Department of Fisheries and Oceans
Fisheries Research Branch
Pacific Biological Station
Nanaimo, British Columbia V9R 5K6

April 1985

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1369

Fisheries and Oceans Pêches et Océans

Canada

85-5619/

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

Numbers 1–456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457–714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715–924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annual des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences No. 1369

April 1985

OBSERVATIONS ON THE FATE OF EXPERIMENTALLY INDUCED WOUNDS ON ADULT CHINOOK SALMON IN SEA WATER

by

G. R. Bell and J. W. Bagshaw

Department of Fisheries and Oceans
Fisheries Research Branch
Pacific Biological Station
Nanaimo, British Columbia V9R 5K6

(c) Minister of Supply and Services Canada 1985

Cat. No. Fs 97-6/1369E ISSN 0706-6457

Correct citation for this publication:

Bell, G. R. and J. W. Bagshaw. 1985. Observations on the fate of experimentally induced wounds on adult chinook salmon in sea water. Can. Tech. Rep. Fish. Aquat. Sci. 1369: 49 p.

ABSTRACT

Bell, G. R. and J. W. Bagshaw. 1985. Observations on the fate of experimentally induced wounds on adult chinook salmon in sea water. Can. Tech. Rep. Fish. Aquat. Sci. 1369: 49 p.

For several years workers in the Southeast Alaska troll fishery have reported finding chinook and coho salmon with assumed non-troll gear wounds before these injuries could be accounted for by the operation of legal net fisheries. The present work was designed to provide estimates of the age of these external injuries to assist in tracing their possible man-made origin.

Chinook salmon (Oncorhynchus tshawytscha) with experimentally induced scrapes and cuts resembling those found in the troll fishery were observed during 5 months post-infliction. Experimental fish were held and fed in fiberglass tanks provided with flowing sea water. Results of descaling were hard to follow because the affected area was difficult to define, and because capture and handling for observation caused further descaling. Induced scrapes such as might be attributable to contact with nets and lines were apparent for about 2 months post-infliction, but had essentially disappeared by 5 months. Similar marks previously reported from troll-caught salmon in Alaska were probably no more than 1 month old. Deep cuts into the musculature were readily visible but no longer raw at 3-4 weeks after wounding. At 5-6 weeks, healing was well advanced but the skin was not yet intact. Histologically, these deep cuts showed several distinguishable phases over the first 6 weeks post-wounding, but thereafter the rate of change was much slower. Extrapolation to the fate of the above types of wounds in wild fish should be made with caution because of the many differences between the experimental and oceanic conditions, and because of the few fish tested. Photographs of gross and histological changes are presented.

Key words: Chinook salmon, Oncorhynchus tshawytscha, sea water, experimentally induced wounds, wound repair, fishing gear wounds

RÉSUMÉ

Bell, G. R. and J. W. Bagshaw. 1985. Observations on the fate of experimentally induced wounds on adult chinook salmon in sea water. Can. Tech. Rep. Fish. Aquat. Sci. 1369: 49 p.

Depuis plusieurs années, des pêcheurs du saumon à la ligne traînante dans les eaux au sud-est de l'Alaska ont signalé des blessures chez des quinnats et des cohos causées par un autre engin qui s'est révélé être des filets exploités dans le cadre d'une pêche légale. La présente recherche vise l'estimation de l'âge du ces blessures externes afin de déterminer leur origine artificielle éventuelle.

Pendant cinq mois, on a observé des saumons quinnats (Oncorhynchus tshawytscha) portant des coupures et des écorchures faites expérimentalement et ressemblant à celles observées chez des saumons capturés à la ligne traînante. Les poissons expérimentaux ont été gardés dans des bacs de fibre de verre à débit d'eau salée. Les résultats de l'enlèvement d'écailles ont été difficiles à suivre parce que la zone touchée était difficile à définir et que la capture et la manutention subséquentes aux fins d'observation ont entraîné la perte d'autres écailles. Les écorchures expérimentales comme celles que pourraient causer des filets et des lignes étaient visibles pendant environ deux mois après avoir été infligées, mais avaient presque disparu après cinq mois. Des blessures semblables signalées chez des saumons capturés à la ligne traînante en Alaska remontaient probablement à un mois. Des entailles profondes dans les muscles étaient facilement visibles trois à quatre semaines après avoir été faites, mais elles n'étaient plus à vif. Après cinq ou six semaines, la cicatrisation était avancée mais la peau n'était pas entièrement régénérée. Au niveau histologique, ces coupures profondes présentaient plusieurs phases reconnaissables au cours des six premières semaines après le début de l'expérience; par la suite, le taux de changement était plus lent. On devra faire preuve de prudence dans l'extrapolation du sort des blessures décrites ci-dessus chez des poissons sauvages à cause des nombreuses différences entre le milieu océanique et les conditions expérimentales et du nombre restreint de spécimens étudiés. Des photographies des changements histologiques et macroscopiques sont aussi incluses.

Mots-clés: saumon quinnat, Oncorhynchus tshawytscha, eau salée, blessures artificielles, cicatrisation, blessures par des engins de pêche

INTRODUCTION

The finding by troll fishermen and processors in Alaska of assumed man-made, non-troll gear marks on chinook (Oncorhynchus tshawytscha) and coho (O. kisutch) salmon before the occurrence of these marks could be accounted for by the operation of domestic net fisheries, led to a plan for a field investigation of these marks and scars in the Southeast Alaska commercial troll fishery (Seibel et al. 1984). In Seibel et al.'s report marks and scars were arbitrarily divided into the following 6 categories:

Category	General Description
1	One or more fairly well delineated linear marks between the head and dorsal fin, approximately perpendicular to the longitudinal body axis and encircling or partially encircling the body.
2	A series of approximately parallel mark or scrape lines over a substantial portion of the body; two or more series of such marks occurring at different angles may give the appearance of cross hatching marks.
3	A fairly well delineated scrape band generally occurring between the head and the dorsal fin approximately perpendicular to the longitudinal body axis or angled slightly backward from the top to the bottom of the body and containing an approximately oval-shaped open wound normally in the upper portion of the body.
4	Extensive descaling of at least 25 percent or more of one or both sides of the body, but with no well delineated marks or wounds.
5	Open, gaping wounds or puncture marks located anywhere on the body either with no other marks and scrapes or with adjacent irregular "scratch" or "claw" marks but no marks as described in Categories 1-4.
6	Any scars/marks not fitting the descriptions in Categories 1-5.

Knowledge of the age of the categories of scars and marks would be of considerable value in determining the source(s) of these afflictions, a question of significance within the context of the deliberations of the International North Pacific Fisheries Commission. The purpose of the experimental investigation reported herein was to provide a basis for estimating how long some of the categories of injuries observed in the field might have persisted. It is recognized, of course, that the experimental conditions differ from the natural conditions, but whether or not these differences lead to significant errors in estimation remains to be seen.

The present report describes the results of observations made over a period of 167 days and provides information on cellular aspects of the repair process.

METHODS

In July 1983, 21 chinook salmon (1-1.5 kg) were taken from seapens and placed in a 2.5 m diameter, 1.2 m deep fibreglass tank supplied with sand-filtered sea water of 28°/$_{oo}$ salinity. The fish were fed daily to satiation with Oregon Moist Pellets (OMP), except on the days before and after sampling.

After a period of adaptation, the fish were anesthetized with tricaine methane sulfonate (MS-222) and cold branded for individual identification. A length of 3 mm cotton twine or a scalpel was then used to scarify each fish in accordance with the description of the particular category chosen. (Categories 1-4 only were chosen for this study because Categories 5 and 6 were considered too indefinite.) Fish were then photographed to record the "0" time or initial condition.

At various times post-scarification fish were captured, anesthetized, and photographed to show the experimentally affected area. The sampling schedule, days since the marks and wounds were inflicted, and water temperatures are shown in Table 1.

HISTOLOGICAL PREPARATION

Category 3 wounds and the surrounding tissues were dissected from certain fish and fixed in Bouin's fluid. Specimens were then processed using standard histological methods and the resulting sections were stained with hematoxylin and eosin (H + E).

RESULTS AND DISCUSSION

There were no mortalities associated with the scarification and handling of the fish but one specimen was lost when it jumped from the tank.

It became extremely difficult, sometimes impossible, to identify individual fish because the brands, approximately 2 cm in size, became obscured. Brands were used principally to incorporate individual identification into the photograph of the scarified area. In view of our experience we would recommend the use of much larger brands or, preferably, any form of readily visible tag. We further recommend that fish be anesthetized prior to removal from the tank, rather than after, as was done

in this work, to reduce the damage due to handling. Anesthesia in a minimum water volume could be accomplished by hypothermia, or probably better, using a chemical anesthetic such as 2-phenoxyethanol.

Observations on the condition of the several categories of marks and wounds induced on various sampling dates are described below. Photographs of the gross appearance are supplemented in the case of Category 3 lesions with photomicrographs.

1. GROSS OBSERVATIONS

Category 1

Several examples of the fate of Category 1 marks are given in Plates I-III. It can be seen that many simulated line or gill-net marks were still visible at 64 days but they had become much less characteristic. Fig. 6 (Plate I) is a good example of the persistence of this category of mark. In other specimens, initial marks were not visible in 64-70 days but this might be attributable to descaling from handling (see Figs. 11 and 12, Plate II; Fig. 18, Plate III).

In general, it appears that Category 1 marks persist recognizably for 60-70 days but become unrecognizable thereafter. The results suggest that Category 1 marks as originally photographed and described in the report on marked troll-caught fish in Alaska (Seibel et al. 1984) had probably occurred within the previous month or less.

Category 2

Although it was possible initially to simulate Category 2 marks, the characteristics did not persist to the next sampling and the marked areas became generally descaled. This progression of events can be seen in Plates IV, V, and VI but particularly in Plate IV where the initial markings resulted in almost complete descaling of the treated area within 21 days (Figs. 20 and 21). By 42-70 days (Figs. 22, 23, 24 and 29) regeneration of scales was becoming apparent but the process appeared to be slow. Further comparison of initial marks with those at 64 days may be made by referring to Figs. 30 and 31.

Poor characterization of this category complicated by inevitable descaling from handling made assessment of repair unsatisfactory. Nevertheless, it seems reasonable to suggest that scale regeneration over an area of the body would take 3 months or more.

Category 3

Plates VI (Figs. 32-35) and VII show the fate of open wounds associated with this Category. These wounds showed a definite healing trend beginning with a covering of the open lesion by clotted blood

(Figs. 33 and 37). Subsequently, the wound began healing from the margins (Figs. 35, 38 and 39) until the original cut was almost repaired by 161 days in the example given (Fig. 40). There were no mortalities of fish inflicted with this type of wound and no gross indications of localized infection.

It is estimated that deep cuts into the musculature would probably take 6 months or so to heal completely and as with mammals would probably leave a permanent scar. It was surprising that the cuts remained so clean and free of progressive decay. Perhaps this would not be the case at sea. Although bacteria did colonize the wound (see Histological observations), the numbers and diversity were probably more limited than they would be in the wild. Further, regular feeding of a nutritious diet such as OMP probably assisted healing. The steady progress of healing suggests that there was adequate provision of dietary Vitamin C, shown by Ashley et al. (1975) to be necessary for repair of this type of wound. However, the foregoing is speculative and one might also argue that fish on a natural diet would be better able to repair wounds.

Category 4

The results of descaling, the only characteristic of this category, proved almost impossible to assess mainly because the initially affected area soon became unrecognizable due to scale loss during handling. There was evidence of scale regeneration at 64 days but under the experimental conditions nothing more definite could be concluded.

2. HISTOLOGICAL OBSERVATIONS

In this aspect of the report, observations have been restricted to those on the open wound featured in Category 3 because this is the most severe type of injury. Descriptions of the healing process are given for 1,3,7,15,21, and 42 days post-scarification, and the area examined is illustrated diagrammatically in Fig. 41. Photomicrographs illustrating this healing process are presented in Figures 42-51, and a key to the lettering can be found near the end of this report.

1 Day - See Figs. 42 and 43

The cut surface was covered by a pale eosinophilic substance (probably congealed tissue exudate [T]) containing large numbers of erythrocytes. A major blood clot (TH) was situated near the centre of the wound beneath which were many damaged and necrotic muscle fibers (M).

At this early post-wounding stage very little evidence of an inflammatory response could be seen at the cellular level, although there were areas of simple hemorrhage deep into the sub-dermal tissues and extending into the musculature.

The edges of the wound were ragged and torn with the epidermis retracted away from the cut, and several scales were sequestered out of position well down into the muscle layers.

3 days - See Fig. 44

The surface of the wound showed the same layer of congealed tissue exudate (T) evident in the 1 day sample, overlying the cut ends of damaged muscle bundles (M).

A vigorous proliferation of inflammatory cells (I) was seen infiltrating the area and macrophages appeared to be active around areas of necrotic tissue (N).

No organized blood clot remained at the surface and as yet no epithelial proliferation appeared to have been initiated from the marginal areas.

Many bacterial cells of several types could be found amongst the necrotizing debris at the open edge of the wound.

7 days - See Figs. 45 and 46.

The exposed surface of the wound was covered by a thin layer (approximately 2-5 m thick) of tissue exudate (T) overlying a thick band of necrotic muscle fibers (N).

A sharp line of demarkation existed between the necrotic tissue (N) and the more deeply situated layers of unaffected muscle (M). Large numbers of erythrocytes accompanied by polymorphonuclear leucocytes and macrophages (I) could be seen infiltrating the tissues adjacent to the damaged areas.

Numerous bacterial cells were still visible in the autolysing debris close to the surface of the wound, and intruding in places deep into the damaged muscle. The lateral edges of the wound showed no discernible change from the earlier samples at 3 days healing time and no epithelialization could be seen.

15 days - See Fig. 47.

During the intervening 8 days intense cellular activity had taken place and the formation of granulation tissue (G) had commenced. However, the sub-dermal areas consisted of a rather chaotic mixture of endothelial cells, fibroblasts, macrophages, and erythrocytes, with partially autolysed muscle cells and collagen (C).

A multi-layered sheet of new epithelium (E) had formed, sealing off the surface and extending down in some areas into the damaged underlying tissues. The basement membrane did not appear to be continuous as would normally be the case in well developed epithelium.

No bacterial cells or other micro-organisms could be seen at this stage of healing.

21 Days - See Fig. 48.

Deep into the damaged area the process of cleaning up the debris by macrophages was well advanced and the formation of new muscle fibers and connective tissue elements had commenced.

Toward the surface, the granulation tissue (G) was still in evidence as was the primitive non-structured type of epithelium (E). However, the granulation tissue was already less vascularized and a more organised regime of fibrous connective tissue and primitive muscle fibers (P) was emerging.

42 Days - See Figs. 49, 50 and 51.

In the central sub-dermal region, the granulation tissue had all but disappeared and strong fibrous elements (F) could be seen filling the gaps between developing muscle and epithelium, and containing well formed blood vessels.

The newly formed epidermis (E) was now much more structured, showing the basal columnar cells, fusiform layer with mucus secreting goblet cells, and the external layer of squamous epithelial cells.

The marginal areas of both areolar (A) and dense fibrous connective tissue (D) in the uninjured, adjoining tissues appeared to be forming anastomoses (J) and becoming continuous with the newly formed tissues of the repairing areas. Scale regeneration had evidently begun as shown by the presence of miniature but well formed scales (S) in the loose connective tissue between the dense fibrous layer and the epidermis.

The histological findings show essentially the same processes of healing and in roughly the same time frame as the wound healing observations of previous investigators.

Finn and Nielsen (1971) did not observe muscle regeneration in rainbow trout over a 16 day period, and Roberts et al. (1973) obtained similar results with salmon as did Mawdesley-Thomas and Bucke (1973) working with goldfish (Carassius auratus). However, Anderson and Roberts (1975) found myofibrillar (muscle) regeneration occurring after 18 days at 23°C and this study showed that muscle regeneration occurred after approximately 21 days at an average temperature of 12°C.

SUMMARY

1. The purpose of this work was to determine the fate of certain grossly visible, experimentally induced external lesions, on salmon held in sea water, with a view to estimating the age of similar marks and wounds on salmon caught by troll gear at sea. Of the four categories of marks

and wounds produced experimentally on adult chinook salmon, only those with well-defined characteristics were useful for this estimation.

2. Net or line marks (Category 1) persisted for about 2 months after infliction, but by 5 months had essentially vanished. Our results suggest that the marks characterizing this category on salmon taken in the Alaska troll fishery (Seibel et al. 1984) had probably been made within a month or less of having been inflicted.

3. Because sampling also caused descaling, almost nothing definitive can be said from this experiment about scale regeneration over an area. Parts of the originally descaled area were still evident after 64 days.

4. Deep cuts into the musculature, characteristic of Category 3, were readily visible, although no longer raw, at 3-4 weeks after injury. At 5-6 weeks, healing was clearly evident but the skin was not yet intact. Our results suggest that the cuts as depicted in the original categorization had probably been made within two weeks of recording.

5. It must be emphasized that estimates of the duration or age of marks made at sea assume a similar rate of healing to that under our experimental conditions. It is possible that the rates could be quite different.

6. The steady progression of the healing process demonstrated histologically and the ability to differentiate between six sampling periods up to six weeks after wounding could be used, with the aid of a series of control slides, as a more sensitive indicator of the age of Category 3 wounds than would the alternative method of gross examination. After approximately two to three months the decreasing rate of change in the histological picture of Category 3 wounds would tend to limit the sensitivity of this method.

ACKNOWLEDGEMENT

We are very grateful to Dr. L. Margolis for his valuable criticism of the manuscript.

REFERENCES

Anderson, C.D. and R. J. Roberts. 1975. A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. J. Fish Biol. 7: 173-182.

Ashley, L. M., J. E. Halver, and R. R. Smith. 1975. Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing, p. 769-786. In W. E. Ribelin and G. Migaki [ed.] The pathology of fishes. University of Wisconsin Press, Madison, Wisconsin, U.S.A.

Finn, J. T. and N. O. Nielsen. 1971. The inflammatory response of rainbow trout. J. Fish Biol. 3: 463-478.

Mawdesley-Thomas, L. E. and D. Bucke. 1973. Tissue repair in a poikilothermic vertebrate, Carassius auratus (L): a preliminary study. J. Fish Biol. 5: 115-119.

Roberts, R. J., A. McQueen, W. M. Shearer and H. Young. 1973. The histopathology of salmon tagging. II The chronic tagging lesion in returning adult fish. J. Fish Biol. 5: 615-619.

Seibel, M., A. Davis, J. Kelly, L. Talley, and P. Skannes. 1984. Observations on externally scarred and marked chinook and coho salmon in the 1982 Southeastern Alaska commercial troll fishery. Informational Leaflet No. 240, Alaska Department of Fish and Game, 71 p.

Table 1. Sampling schedule of fish marked and wounded according to each of the four categories tested.

Date	Days post-scarifying		No. of fish in category				T (°C)
	Fish 1-7	Fish 8-21	1	2	3	4	
July 29, 1983	0		3	2	2		11.6
Aug. 4, 1983		0	5	3	3	3	12.2
Aug. 19, 1983	21	15					12.0
Sept. 9, 1983	42	36					11.7
Oct. 7, 1983	70	64					11.3
Jan. 12, 1984[1]	167	161					6.0

[1]Date of last observation.

CAPTIONS TO PLATES AND FIGURES

Plate I

Fig. 1. Category 1, fish #09. 0 days post-injury. (Identity card $2cm^2$).

Fig. 2. Category 1, fish #09. 0 days post-injury.

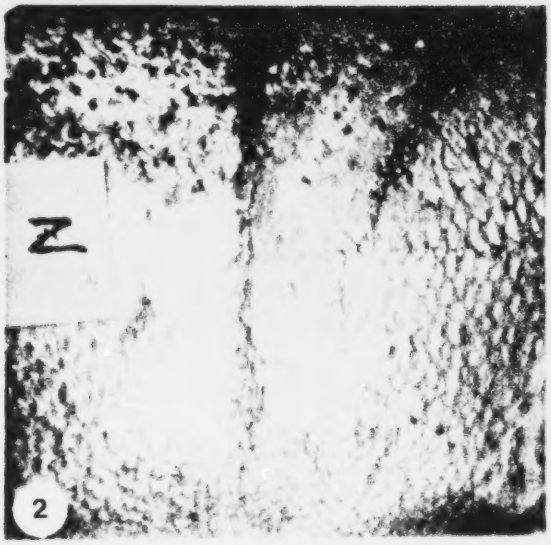
Fig. 3. Category 1, fish #09. 15 days post-injury.

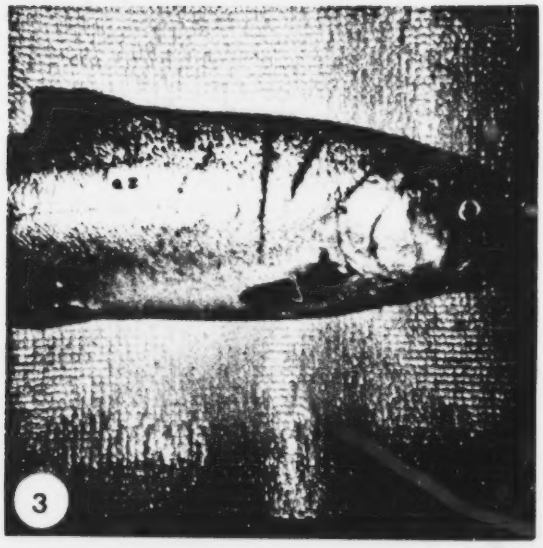
Fig. 4. Category 1, fish #09. 15 days post-injury.

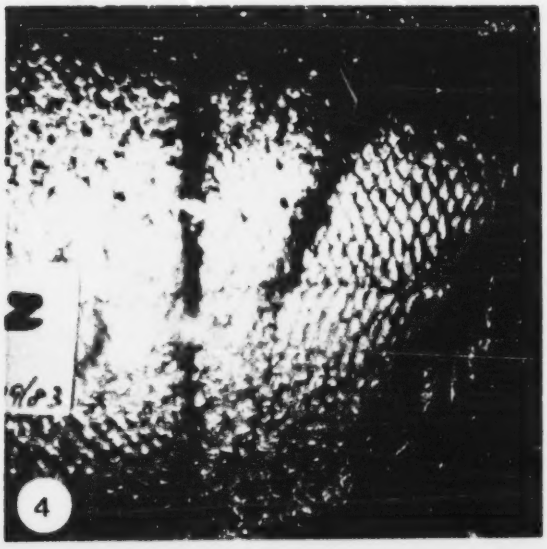
Fig. 5. Category 1, fish #09. 36 days post-injury.

Fig. 6. Category 1, fish #09. 64 days post-injury.

1


2


3


4

5
7 SEPT. 0

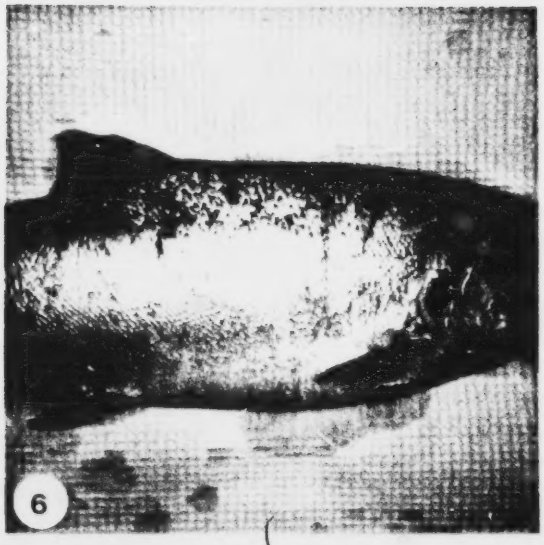

6

Plate II

Fig. 7. Category 1, fish #08. 0 days post-injury.

Fig. 8. Category 1, fish #08. 15 days post-injury.

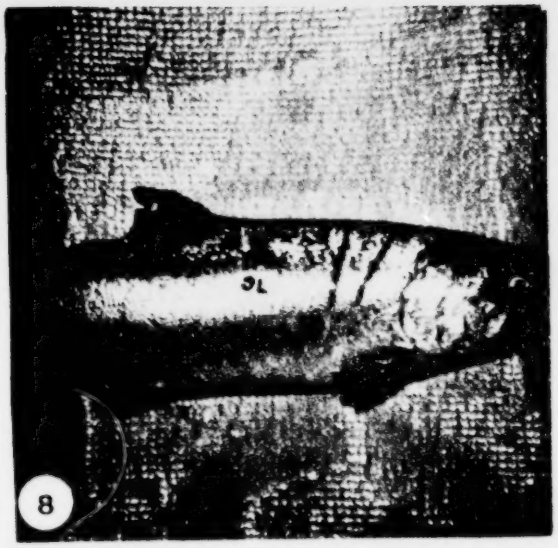
Fig. 9. Category 1, fish #08. 15 days post-injury.

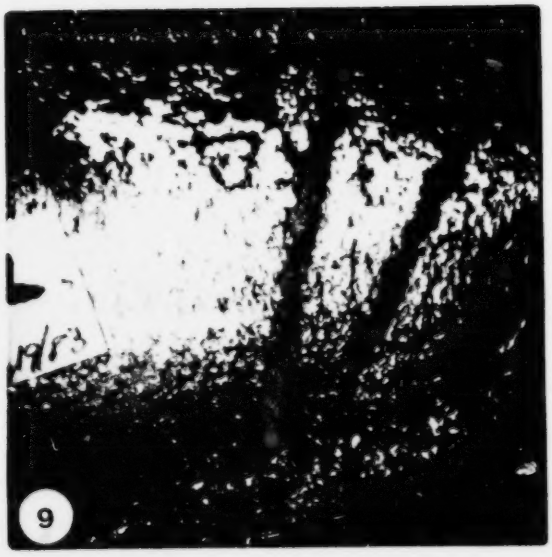
Fig. 10. Category 1, fish #08. 36 days post-injury.

Fig. 11. Category 1, fish #08. 64 days post-injury.

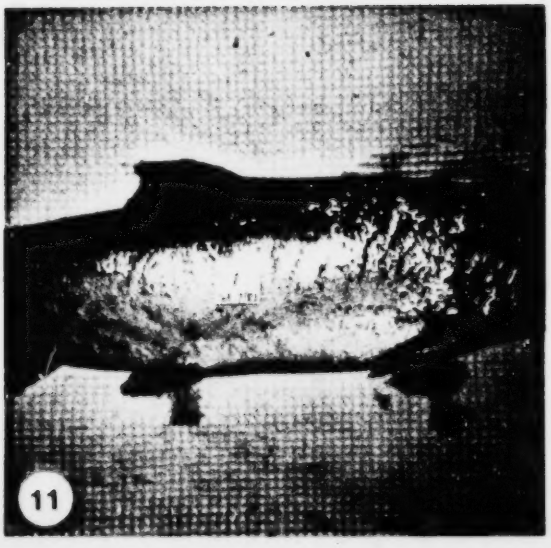
Fig. 12. Category 1, fish #08. 161 days post-injury.

CL
7


8


9

10


11

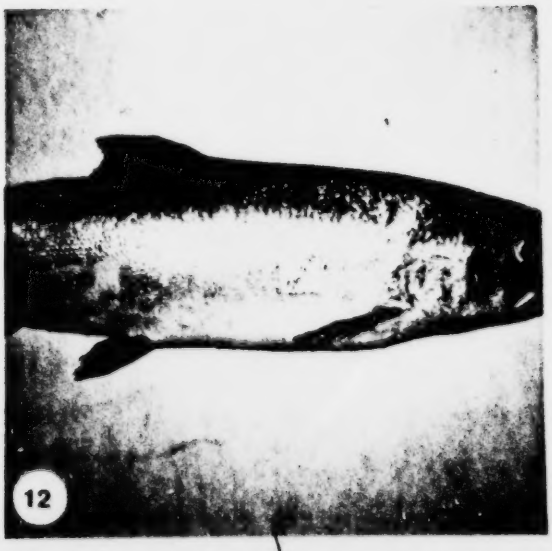

12

Plate III

Fig. 13. Category 1, fish #01. 21 days post-injury.

Fig. 14. Category 1, fish #01. 21 days post-injury.

Fig. 15. Category 1, fish #01. 42 days post-injury.

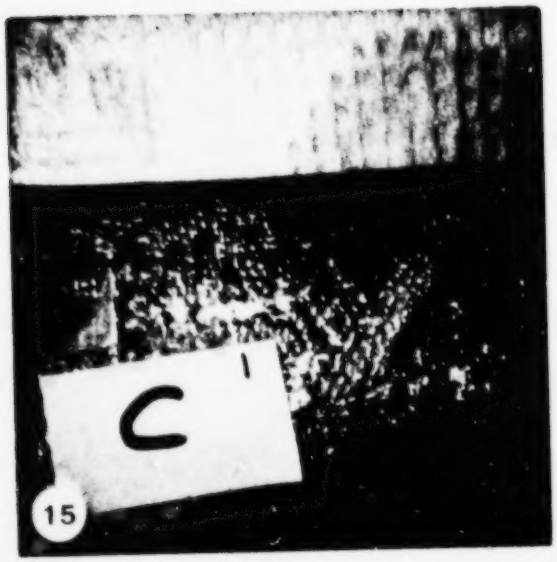
Fig. 16. Category 1, fish #01. 70 days post-injury.

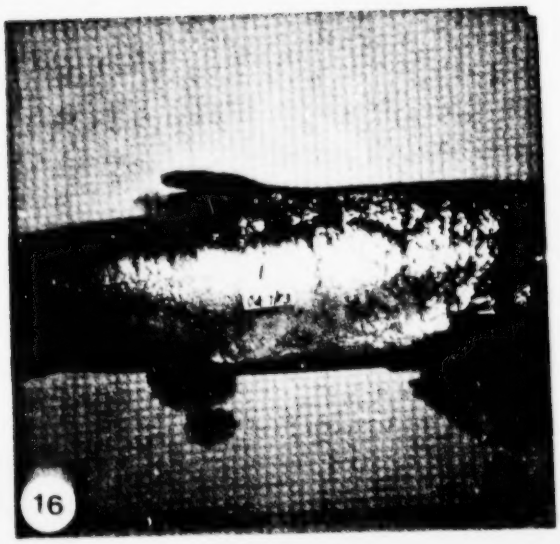
Fig. 17. Category 1, fish #02. 21 days post-injury.

Fig. 18. Category 1, fish #02. 70 days post-injury.

13

14


C
15


16

17

18

Plate IV

Fig. 19. Category 2, fish #05. 0 days post-injury.

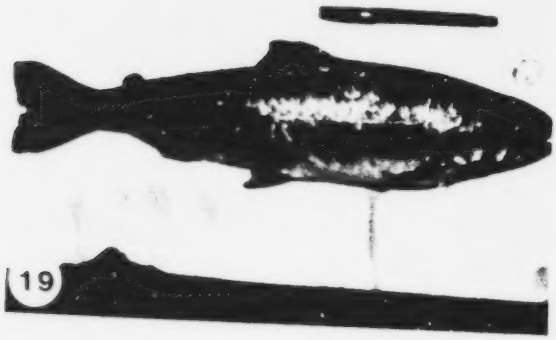
Fig. 20. Category 2, fish #05. 21 days post-injury.

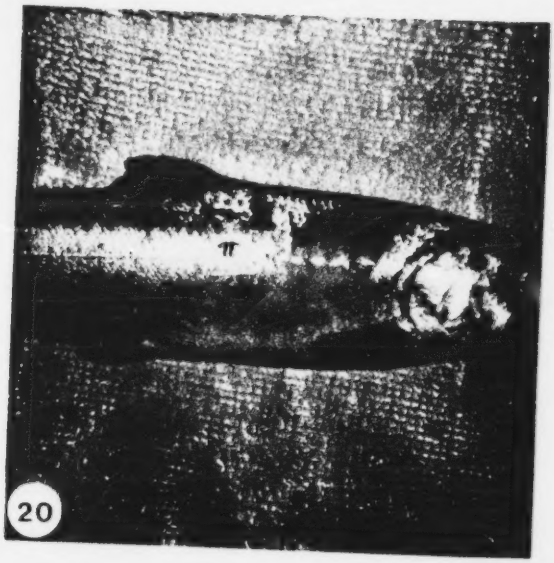
Fig. 21. Category 2, fish #05. 21 days post-injury.

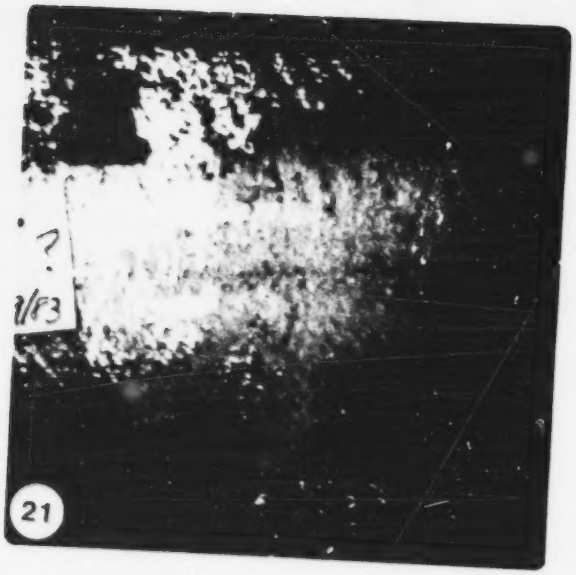
Fig. 22. Category 2, fish #05. 42 days post-injury.

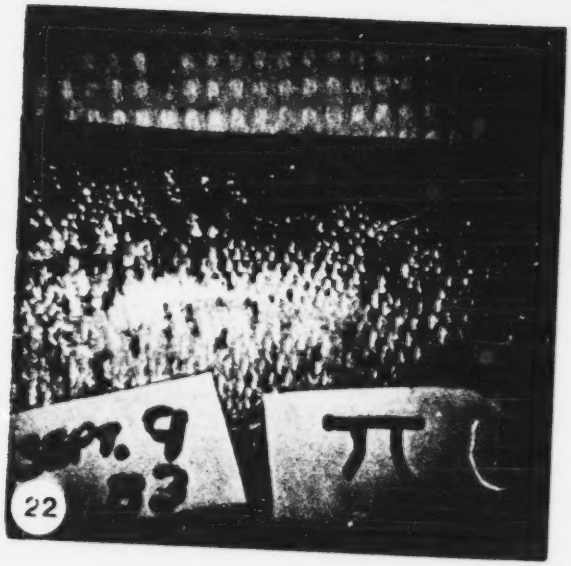
Fig. 23. Category 2, fish #05. 70 days post-injury.

Fig. 24. Category 2, fish #05. 70 days post-injury.


19


20


21


22

23

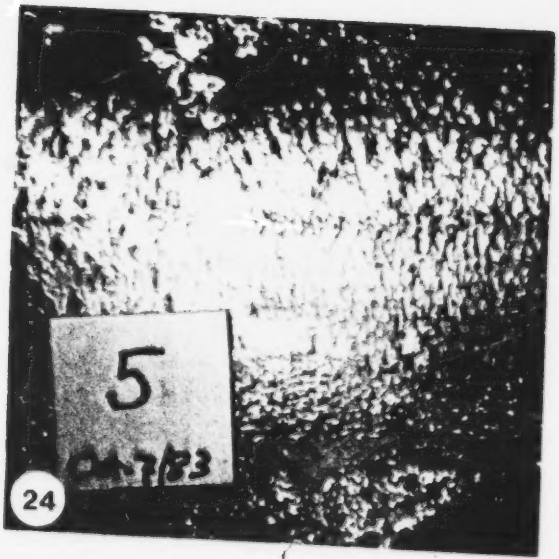

5
24

Plate V

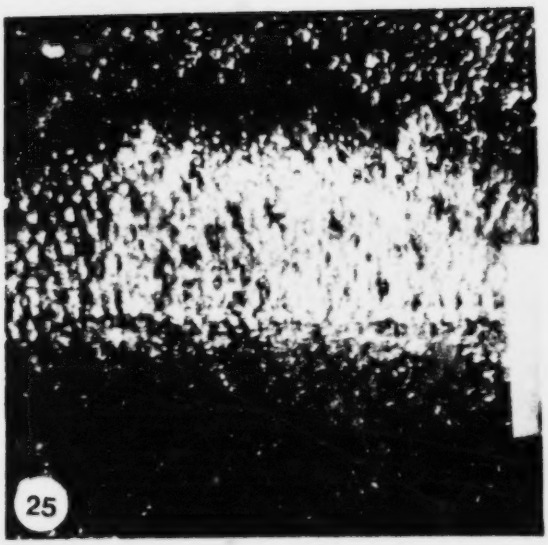
Fig. 25. Category 2, fish #11. 0 days post-injury.

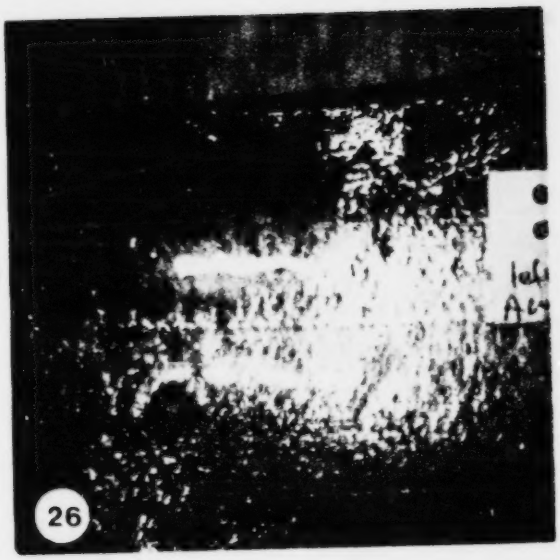
Fig. 26. Category 2, fish #11. 15 days post-injury.

Fig. 27. Category 2, fish #11. 15 days post-injury.

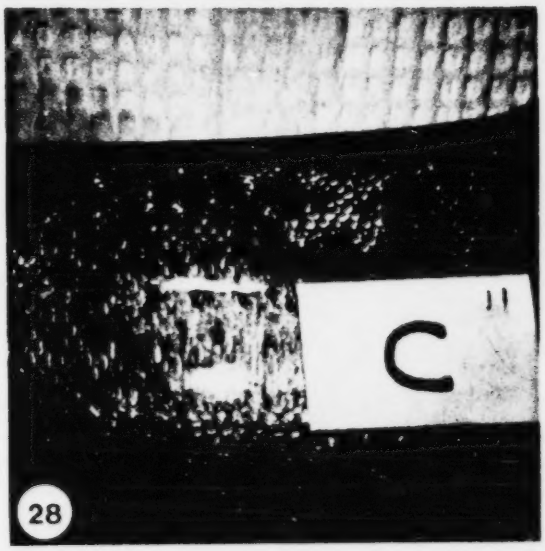
Fig. 28. Category 2, fish #11. 36 days post-injury.

Fig. 29. Category 2, fish #11. 64 days post-injury.


25


26

27


C
28

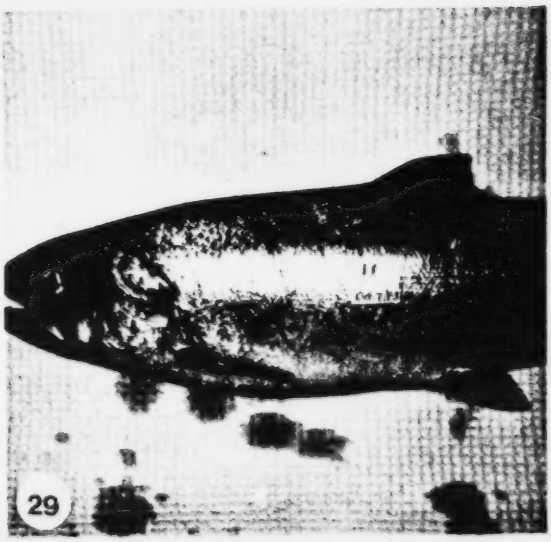

29

Plate VI

Fig. 30. Category 2, fish #12. 0 days post-injury.

Fig. 31. Category 2, fish #12. 64 days post-injury.

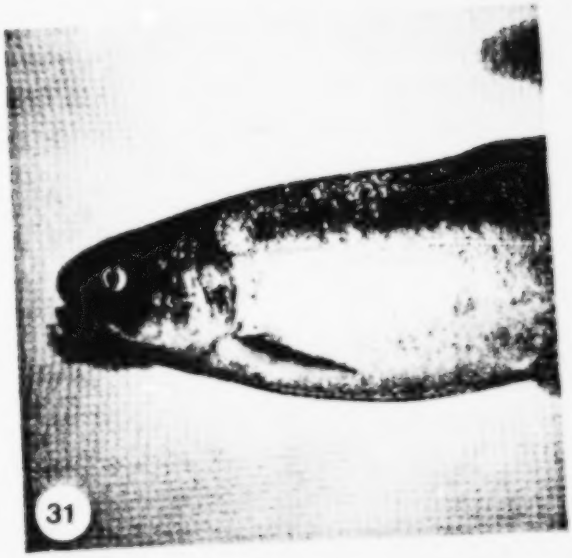
Fig. 32. Category 3, fish # (random). 7 days post-injury.

Fig. 33. Category 3, fish #06. 21 days post-injury.

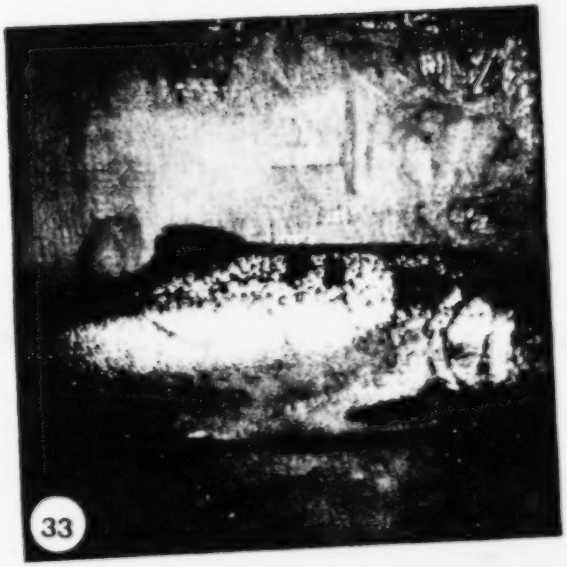
Fig. 34. Category 3, fish #06. 40 days post-injury.

Fig. 35. Category 3, fish #06. 40 days post-injury.

30


31

32


33

34

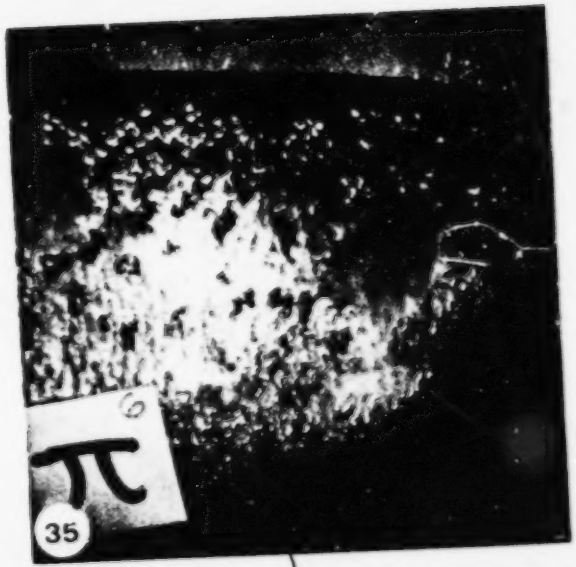

35

Plate VII

Fig. 36. Category 3, fish #16. 0 days post-injury.

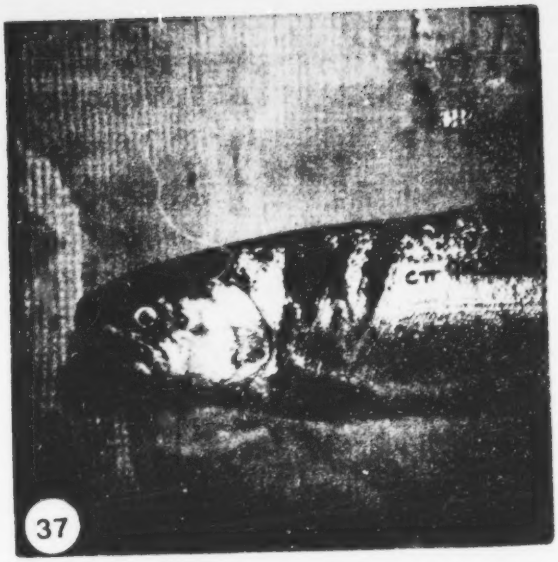
Fig. 37. Category 3, fish #16. 15 days post-injury.

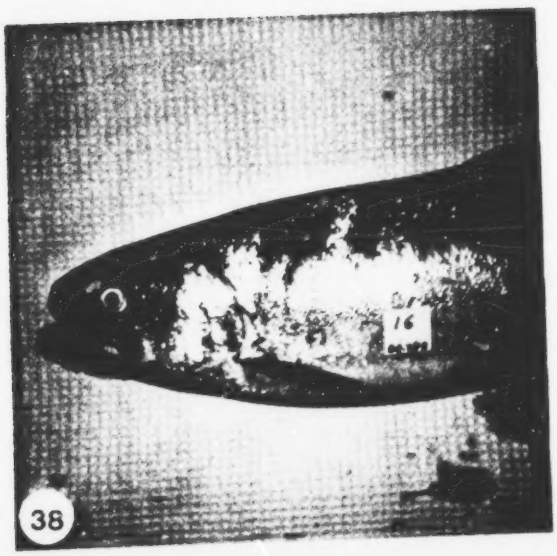
Fig. 38. Category 3, fish #16. 64 days post-injury.

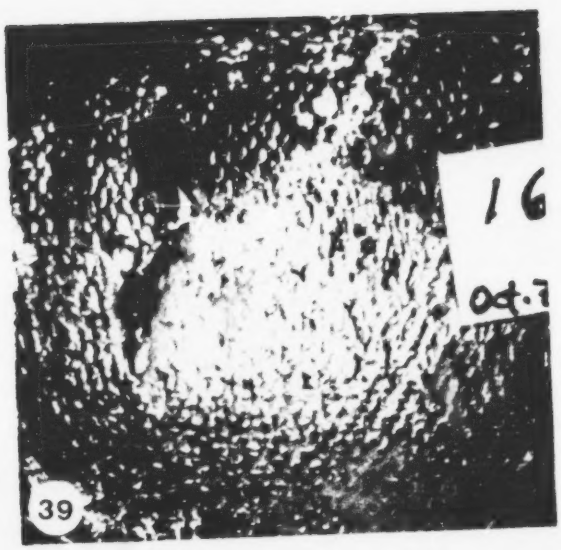
Fig. 39. Category 3, fish #16. 64 days post-injury.

Fig. 40. Category 3, fish #16. 161 days post-injury.

CII
36


CII
37


16
38


16
39

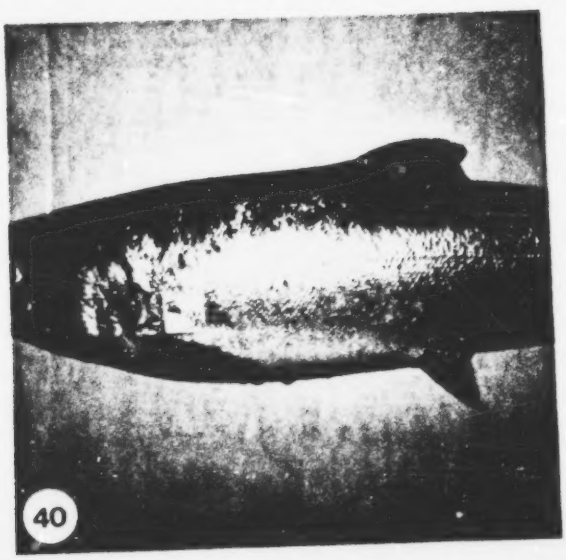

40

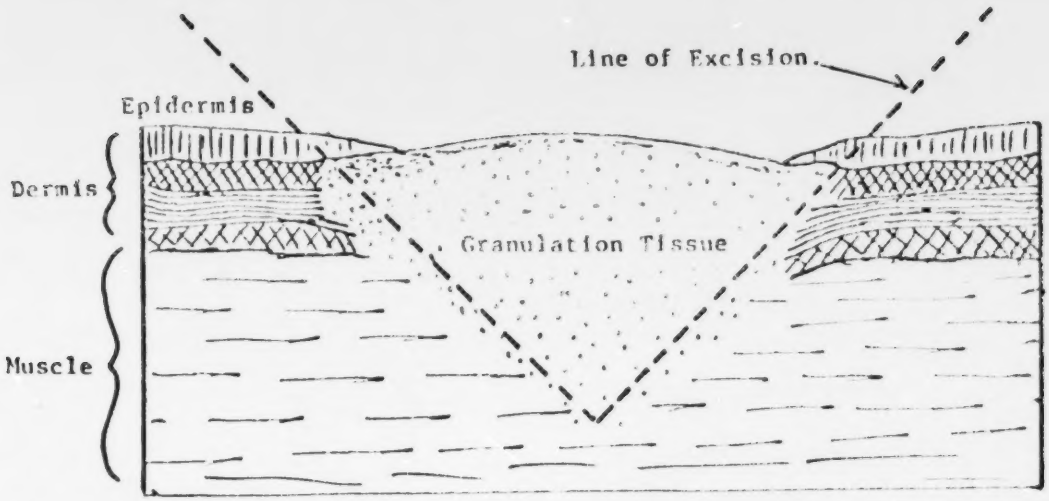
Plate VIII

Fig. 41. Diagram of category 3 wound showing relative orientation of tissues.

Fig. 42. Photomicrograph of category 3 wound. 1 day post-injury. H + E. X 32.

Fig. 43. Photomicrograph of category 3 wound. 1 day post-injury H + E. X 100.

VIII


Line of Excision
Epidermis
Dermis
Granulation Tissue
Muscle

41

42

43

Plate IX

Fig. 44. Photomicrograph of category 3 wound. 3 days post-injury. H + E. X 100.

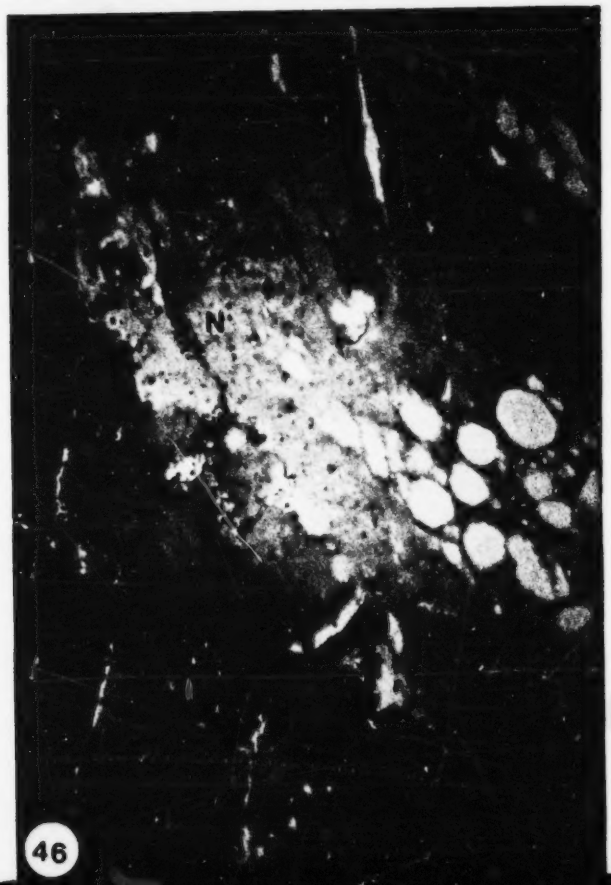
Fig. 45. Photomicrograph of category 3 wound. 7 days post-injury. H + E. X 32.

Fig. 46. Photomicrograph of category 3 wound. 7 days post-injury. H + E. X 100.

Fig. 47. Photomicrograph of category 3 wound. 15 days post-injury. H + E. X 32.

44

45



46

47

Plate X

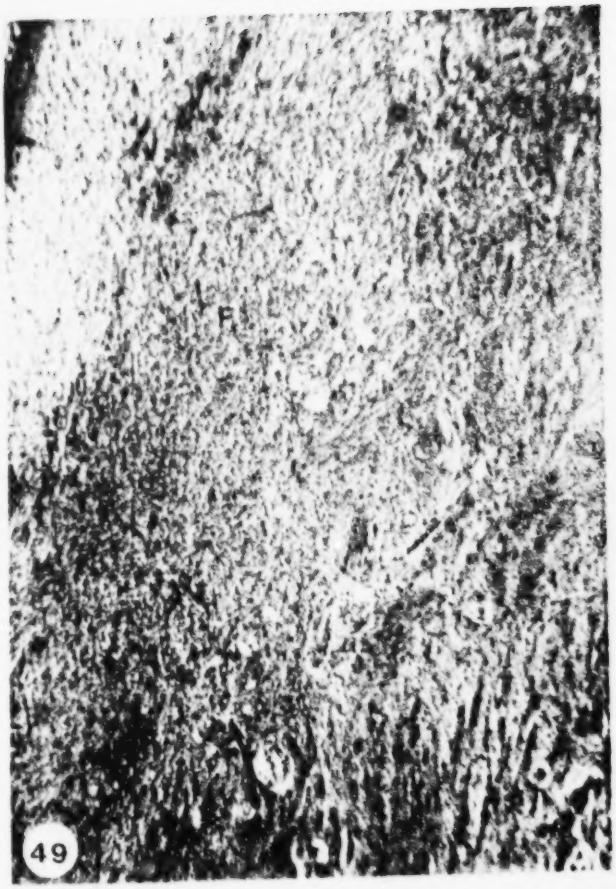
Fig. 48. Photomicrograph of category 3 wound. 21 days post-injury. H + E. X 32.

Fig. 49. Photomicrograph of category 3 wound. 42 days post-injury. H + E. X 32.

Fig. 50. Photomicrograph of category 3 wound. 42 days post-injury. H + E. X 32.

Fig. 51. Photomicrograph of category 3 wound. 42 days post-injury. H + E. X 100.

48


49

50

51

KEY TO LETTERING ON PHOTOMICROGRAPHS

BLOOD CLOT	TH
CONGEALED TISSUE EXUDATE	T
MUSCLE	M
INFLAMMATORY CELLS	I
NECROTIC TISSUE	N
GRANULATION TISSUE	G
EPITHELIUM/EPIDERMIS	E
PARTIALLY AUTOLYSED MUSCLE AND COLLAGEN	C
PRIMITIVE MUSCLE FIBRES	P
STRONG FIBROUS ELEMENTS	F
AREOLAR TISSUE	A
DENSE FIBROUS CONNECTIVE TISSUE	D
ANASTOMOSING AREA	J
SCALE	S